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IMPACT OF HEAVY METAL POLLUTION ON HAEMATOLOGICAL PARAMETERS IN FRESHWATER FISHES

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ABSTRACT

In the present investigation, an attempt has been made to evaluate the effect of pollutants on the hematological parameters of freshwater fishes from the polluted site of Varahanadhi River. The parameters determined were total erythrocyte count (RBC Count), hemoglobin (Hb), mean corpuscular volume (MCV), Mean Corpuscular hemoglobin concentration (MCH) and total leucocyte count (WBC). The above mentioned parameters revealed variations when compared with the control. The alterations in these parameters have ultimately become the causative factor, affecting the general health status and hampering the entire metabolic mechanism of the fishes.

KEYWORDS: Fishes, Hematology Studies, Varahanadhi River & MCV

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INTRODUCTION

Intensification of human population is accompanied with industrialization, resulted in the increasing pollution in the aquatic ecosystems. Water gets polluted by industrial waste materials (petroleum, dyes, detergents and heavy metals), agricultural insecticides and surplus fertilizers, natural and domestic wastes, lead, mercury and sewage that are released into water. It influences the aquatic life and the humans through the food chain.

Hematological parameters are used as indicators of disease or stress due to pollutants and environmental fluctuations. With increasing emphasis on pisciculture and greater awareness of the pollution of natural water resources, hematological studies in fish have assumed greater significance. Stress is a general and non- specific response to any factors disturbing homeostasis. It involves various physiological changes, including alteration in blood composition and immune mechanism (Svoboda, 2001; Witeska, 2003). Stress in fish may be induced by various abiotic environmental factors (changes in water, temperature, pH, O_2 concentration and pollution). Hematological parameters are related to the response of the animal to the environment, where fishes live could exert some responses to the stressors and thus their health. Hematological characteristic indices have been effectively employed in monitoring status under such adverse conditions (Gabriel, 2004).

Hematological Parameter (Blood) of the fish acts as an indicator of physiological and pathological changes in fishes. Blood in the gill has direct contact with water medium and any unfavorable change in the water might intervene the functions of the circulatory system(Cazenave, 2005). They are closely related to the response of the animal to the environment, such as hypoxia, anaesthetic and acclimation (Akinrotismi, 2009). Metals in hematological parameters of fish generally occur due to the osmotic changes resulting in hemo dilution or hemo concentrations (Tort, 1988). Changes in hematological parameters depend upon the aquatic biotope, fish species, age and sexual maturity and health status (Patriche, 2011). Blood constitutes about 1.3-7% of the total body weight

of fish and is one of the most active components of the hematopoietic organs, which contributes to metabolism through gaseous exchange between the organisms and the environment. Thus, blood parameters are used as indicators of the physiological condition or sub lethal stress response in fish to endogenous or exogenous (Belanger, 2001). The aim of the study is to reveal the physiological condition or sub lethal stress response in fishes from Varahanadhi River, by its hematological parameters.

MATERIALS AND METHODS

Varahanadhi River (Villupuram District, Tamil Nadu, India) lays 12.040' N latitude and 79.304' E longitude. It covers a total area of 798 ha and covers within the survey of India, with a total catchment area of about 21 Km. Varahanadhi basin is one of the major basins located in Villupuram, Thiruvavannamalai, Kancheepuram and Cuddalore districts of TamilNadu and Pondicherry. It is surrounded by Bay of Bengal in the east, Palar basin and Nallavur sub basin in the north and Ponnaiyar basin in the south-west. The present study was carried out at different sites. Site A (upstream) which is situated in Gingee town and has its source in the hills of Melmalayanur in the South Arcot District of Tamil Nadu. Site B (midstream) is the branch of the river that flows through Villupuram District and site C (downstream) part of the river called Sankaraparani that drains into the Bay of Bengal. The chemicals used for the analysis were of analytical grade (Merck, India).

Sampling and analysis were carried out according to standard methods prescribed by WHO in freshwater fishes (catla and mrigal). Collection site includes: upstream, midstream and downstream in Varahanadhi river located in Villupuram district and control fishes were the fishes maintained in the laboratory.

Five fishes of each species were collected from polluted site and were transported in polythene bags, half filled with water, without any disturbance. About five fishes were put in each bag with water, the bags were aerated using pressurized air flow from a cylinder. This mode of transport was successful, since there was no mortality in all consignment throughout the course of this study. The samples were brought to the laboratory on the same day.

Fish samples were measured and weighed (15 ± 5cm and 150-200g).

COLLECTION OF BLOOD

The fish was cleared off water from the body surface, then the caudal peduncle was severed and the blood was collected using a disposable syringe from the caudal artery and transferred into heparinised tubes. RBC count was done with a Neubauer crystalline counting chamber as described by Sohn Henry *et al.*,(1969). WBC count was determined following the procedure by Hunter and Bomford *et al.*,(1963). Hemoglobin concentration (Hb) was estimated using cyano-methemoglobin method (Blaxhall *et al.*,1973). The red cell indices:mean corpuscle volume MCV, mean corpuscle hemoglobin (MCH) and mean corpuscle hemoglobin concentration (MCHC) were calculated using standard formulas (Coles*et al.*,1986) and PCV (Miale *et al.*, 1982).

The data obtained were collated and analyzed with analysis of variance (ANOVA), where differences existed, mean separation was done with Turkey HSD test at 0.05% probability (Wahua *et al.*, 1999).

RESULTS AND DISCUSSIONS

Water quality is one of the major factors responsible for individual variation in fish hematology. Since they are sensitive to slight fluctuation that may occur within their interval milieu (Fernandes *et al.*, 2003). The parameters examined

in this study, indicated values, characteristics of fresh water environment and all the parameters were in a tolerable range. The knowledge of the hematological characteristics is an imperative means that can be used as an effective and sensitive index to monitor physiological and pathological changes in fishes.

Figure 1: Images of Catla and Mrigal fishes collected for the study

RBC Count and Hemoglobin Content

The RBC count and Hemoglobin content decreased (Figure 1). Such decrease may be due to damage and destruction of blood cells (Hardy *et al.*, 1973). Changes in blood parameters are caused by stressors and pollutants (Agarwal *et al.*,1976). The reduction in RBC Count might be due to hemolysis or erythropoietic disorders that leads to anaemic condition in fish. The anaemic condition in fishes is attributed to an inhibition on erythrocyte production or haemodilution (Larson *et al.*, 1975). Similar results with RBC and hemoglobin content reduction was reported with fishes exposed to different heavy metals (Karuppasamy *et al.*, 2005).

Hemoglobin level was found to be decreased (Figure 2)and the anaemic response could be as a result of disruption in erythrocyte production (Omoregie *et al.*, 1995), haemodilution (Sampath *et al.*, 1993) and destruction of intestinal cells involved in the production of vitamin B_{12} used in the production of the hemoglobin portion of red cells (Gardner and Yenich 1970). Hemoglobin level was found to be reduced due to pollution, caused by heavy metals and lose their oxygen binding property, which resulted in erythrocyte damage (Witeska *et al.*, 2003). Anaemic condition may be due to an increased rate of erythrocyte destruction in hemopoietic organs (Jenkins *et al.*, 2003; Seth and Saxena, 2003).

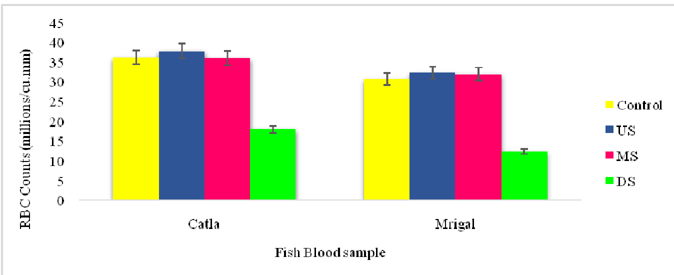

Figure 1: Estimation of RBC Count in Fish Blood Samples

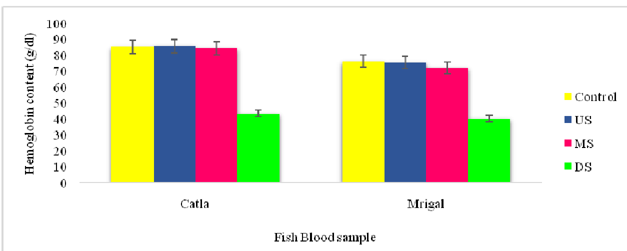

Figure 2: Estimation of Hemoglobin Content in Fish Blood Samples

WBC Count

WBC count is found to be increased in the present study which is considered as an adaptive mechanism (Figure 3). This may be due to the direct stimulation of the immunological defense mechanism against stressor (Henry *et al.*, 1978). Such increase in WBC count may be due to lymphocytosis and immune response (Shah SL *et al.*, 2005). The increase in number of WBC's may plays an important role in immunological defense systems during exposure to toxicants like heavy metals and appears to be associated with circulatory levels of granulocytes, which are known to respond for phagocytosis (Briton*et al.*, 1963, Kori – Siakpere and Ubogu 2008). Alteration in hematological parameters are not only associated with physico-chemical properties of water. But also due to season, reproductive activities and sex (Pandey *et al.*, 1977; Banerjee *et al.*, 1999), photoperiodism (Srivastav *et al.*, 2010; Valenzuela *et al.*, 2006) and availability of food (Diana *et al.*, 1990). Similar reports were given by Murugesan *et al.*, (1985).

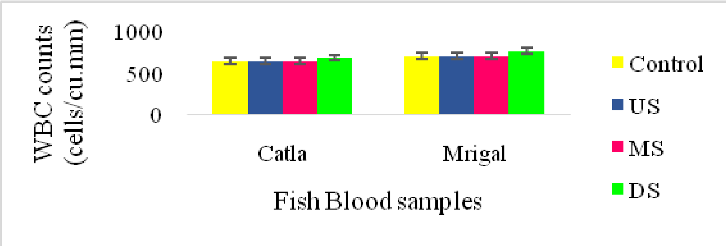

Figure 3: Estimation of WBC Count in Fish Blood Samples

MCH, MCV & MCHC

An increasing trend in the mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCH) and mean corpuscular hemoglobin concentration (MCHC) were seen in fishes from the polluted site when compared with the control. (Table 4). These hematological disturbances are hemopoietic or erythrocyte mobilization response to the heavy metal induced stress. Karuppasamy (2005), reported similar reduction in MCH values with heavy metal pollution. These hematological disturbances are a hemopoietic or erythrocyte mobilization response to hypoxemia induced by heavy metal stress.

Table4: Estimation of MCV, MCH and MCHC in Fish Blood Samples (Mean ± S.D)

Sample Collection Area	MCV (fl)		MCH (pg)		MCHC (g/dl)	
	Catla	Mrigal	Catla	Mrigal	Catla	Mrigal
Control(lab maintained)	50.83±0.10	42.16±0.19	17.83±0.07	14.66±0.05	32.00±0.14	31.67±0.41
US	49.17±0.45	41.00±0.09	17.33±0.20	14.33±0.09	31.83±0.84	30.39±0.22
MS	47.17±0.25	39.00±0.05	16.00±0.26	13.00±0.23	31.33±0.14	30.89±0.19
DS	80.50*±0.18	71.33*±1.61	10.16*±0.18	05.17*±0.25	26.50*±1.32	23.83*±0.38

Key: * Significant values, US- Upstream, MS- Midstream and DS- Downstream. MCV- Mean corpuscular volume, MCH- Mean corpuscular Hemoglobin, MCHC- Mean corpuscular Hemoglobin concentration, PCV- Packed cell volume. Mean are significantly different at 0.05% (p>0.05)

The changes in MCH and MCHC may be either due to the (i) prevalence of large number of small sized immature RBCs in general circulation or even (ii) reduction in cellular blood iron thereby resulting in reduced Hb synthesis. Thus

decline or fluctuating pattern of MCH and MCHC definitely appears to be the reflection of marked decline in Hb, because of chronic toxicity of zinc and sevin (Raina *et al.*, 2014). MCV, MCH and MCHC were calculated indirectly with reference to RBC, Hct and Hb. Therefore, their changes are directly linked with these blood parameters (Fazio *et al.*, 2013). In response to respiratory difficulty, the organism stimulates an increase in RBCs, Hb and MCHC as mechanism to enhance oxygen transfer (Affonso *et al.*, 2002).

Packed Cell Volume (PCV)

The low value of PCV (Hematocrit value) in fish was observed (Table 5). Fish exposed to stress was attributed to a reduction in RBC volume caused by osmotic changes (Aiwan *et al.*, 2009). Similar reports were given by Majid Tak *et al.*, (2014) in *Cyprinus carpio*. The hematological changes during the entire period in Hb, RBCs and PCV, this reveals the prominent anemic effect of edifenphos which is confirmed by the results of blood indices and may explain the chocolate discoloration of parenchymatous organs, as hemoglobin may be converted into methemoglobin with resultant hemolysis and reduced blood oxygen carrying capacity, which accumulates with the irritant effect of edifenphos causing respiratory distress to the fish. The severity of the anaemia is also magnified by the hypoproteinemia effect showed by edifenphos (Gaafar *et al.*, 2010).

Table 5: Estimation of PCV in Fish Blood Samples (Mean ± S.D)

PCV (%)	Catla	Mrigal
Control	24.50±0.30	21.33±0.23
US	24.10±0.23	20.33±0.58
MS	24.50±1.96	21.00±0.27
DS	08.83*±0.80	04.10*±0.34

Similar reports were given by Kandari *et al.*, (2015) in Lacepede for hematological parameters in seasonal background. Other reports of Senthemilselvan *et al.*, (2012) in *Latus calcarifer*, Vazquez *et al.*, (2007) in *Cichlasoma dimerus*, Ishikawa *et al.*, (2007) and Gaafar *et al.*, (2010) supported the concepts of this present study on hematological parameters. Whereas, Bhatkar *et al.*, (2011) in *Labeo rohita* and Dutta *et al.*, (2015) in Bloch gave contrast result in WBC estimation and gave their reasons.

CONCLUSIONS

Fish blood components indicate the harmful effects of water quality and therefore ensures safety food for people and act as the biological indicator of aquatic pollution. Hematological indices of fishes are closely related to the aquatic environment and the blood will expose conditions within the body of the fish earlier than any visible sign of disease. Analysis of the biochemical, enzymatic and marker analysis will refine the study to a great consideration in the downstream area of the river.

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